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Gold nephropathy: serologic data suggesting an immune complex disease

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SUMMARY

Serological tests performed on a patient with gold-induced nephropathy revealed a multitude of immunological phenomena preceding the onset of proteinuria. These included formation of tissue antibodies, rheumatoid factors and circulating immune complexes. An antigen sharing immunological determinants with DOC-extractable tissue antigens was released into circulation before and during proteinuria. Precipitating antibodies against this circulating antigen were found in one serum sample obtained 6 weeks before the complication was diagnosed. In this serum specimen, antibodies were also found which combined with saline extracts, DOC extracts containing the ubiquitous tissue antigen (UTA), and preparations obtained from various human organs by extraction at 100°C followed by precipitation at 71% ethanol concentration (BE preparations). Most of the activities disappeared before the onset of proteinuria. The possible significance of these phenomena in the pathogenesis of nephropathy is discussed.

INTRODUCTION

Gold salts have been used for treatment of rheumatoid arthritis for more than four decades (Empire Rheumatism Council, 1960), and recently also for the treatment of pemphigus (Penneys *et al.*, 1973). Unfortunately, chrysotherapy is often accompanied by various side effects, the most serious one of which is an immune complex-type glomerulonephritis (Silverberg *et al.*, 1970). The glomerular lesions are pathologically and ultrastructurally indistinguishable from the findings in the idiopathic membranous nephropathy (Silverberg *et al.*, 1970; Ehrenreich & Churg, 1968; Törnroth & Skrifvars, 1974). The specificity of the antigens and antibodies responsible for this complication of gold therapy is not known. In this communication we describe a patient who received gold for the treatment of pemphigus and developed a nephrotic syndrome 4 months after the therapy was initiated. A multitude of immunological phenomena preceded the onset of proteinuria, including formation of tissue antibodies, circulating immune complexes and anti-immunoglobulins.

CASE REPORT

M.M., a 41-year-old Caucasian male, had had pemphigus vulgaris for 11 years. The diagnosis was made by the following findings: (a) clinically: superficial, fragile bullae, involving the mouth, face and trunk; (b) histopathologically: demonstration of acantholytic cells in blister biopsies and deposition of IgG in the squamous intercellular spaces; and (c) serologically: demonstration in the serum of pemphigus antibodies (Beutner & Jordan, 1964) in an initial titre of 640. The patient was treated with 40–80 mg of prednisone/day during the first 8 years with only partial control of his disease. He, however, developed marked Cushingoid features and X-ray evidence of bilateral aseptic necrosis of femoral heads secondary to the steroid treatment. Intramuscular methotrexate, 50 mg/day, was then added to prednisone in the

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TABLE 1. Serologic findings in fifteen sequential sera from patient M.M. with gold nephropathy

Serum number and date	Anti-DOC extract antibody*	Circulating serum antigen†	Latex-agglutination (1:20)	Waaler-Rose test titre	'Ripley' test titre	Platelet aggregation (Pl.A.) titre	Sedimentation character of Pl.A. component	Proteinuria (g/24 hr)
1. 16 November 1973	–	+	–	< 10	20	< 10		0
2. 19 December 1973	–	++	–	20	40	< 10		
3. 2 January 1974	–	–	–	40	80	< 10		
4. 30 January 1974	+++	–	+	320	< 10	640	Heavy¶ and light§	
5. 7 February 1974	–	++	–	160	< 10	40		
6. 13 March 1974	–	+	–	< 10	20	80	Intermediate**	2+‡
3 April 1974								2·9
1 May 1974								4·4
7. 15 May 1974	–	–	–	< 10	40	40		2·2
8. 3 July 1974	–	++	–	< 10	20	< 10		1·75
9. 14 August 1974	–	–	–	40	40	< 10		
10. 14 September 1974	–	–	–	< 10	160	< 10		0·27
11. 23 October 1974	–	–	–	< 10	40	< 10		
12. 22 November 1974	–	–	–	< 10	40	160	Intermediate (7S–19S)	
13. 28 December 1974	–	–	–	< 10	40	< 10		
14. 5 February 1975	–	–	–	< 10	40	< 10		
15. 24 March 1975	–	–	–	< 10	160	< 10		0

* Tested by DDGP.
† Tested by DDGP with serum No. 4.
‡ Albustix (Ames Div. Miles Laboratories, Elkhart, Ind.).
¶ $\geqslant$ 195.
§ $\leqslant$ 7S.
** 75–19S.

hope of eventually reducing steroid dosage, but had to be discontinued because of worsening of the stomatitis. Azathioprine (150 mg/day) was then employed together with prednisone (40 mg/day) but during a 1½-year trial the patient persisted in developing vegetating pemphigus lesions.

Gold therapy in the form of weekly i.m. injections of 30–50 mg of gold sodium thiomalate (Solganol) was begun on 16 November 1973. Azathioprine (150 mg/day) and prednisone (40 mg/day) were also continued. On 27 March 1974, after the patient had received a total of 665 mg of gold preparation, he was noted to develop proteinuria and the therapy was discontinued. Significant proteinuria persisted for 3 months (Table 1). Six weeks prior to the development of proteinuria the patient ceased to have any oral or cutaneous pemphigus lesions. Daily prednisone had been gradually reduced from 40 to 15 mg and azathioprine from 150 to 100 mg. Despite discontinuation of the gold therapy, the patient remained in clinical remission until August 1974, when pemphigus lesions re-occurred. His pemphigus activity was then controlled with daily azathioprine (150 mg) and prednisone (60 mg). At present (December 1975) the patient is free of disease receiving daily azathioprine (100 mg) and prednisone 15 mg).

MATERIALS AND METHODS

Serological tests

Double diffusion gel precipitation (DDGP) tests were performed utilizing 0·5% agarose (SeaKem, MCI Biomedical, Maine) poured onto 50 × 12 mm disposable Petri dishes (Falcon Plastics, Div. Becton, Dickinson & Co., Oxnard, California). Thimerosal at a final concentration of 0·01% was added into agarose as a preservative. Diffusion was allowed to take place for 24 hr at room temperature followed by 48–72 hr at 4°C.

Immunoelectrophoresis tests were carried out in 1% agarose dissolved in Tris–Barbital–Sodium Barbital buffer (Gelman Instrument Co., Ann Arbor, Michigan), ionic strength 0·06, pH 8·8. Samples were electrophoresed at 250 V, 5–10 mA, for 60 min at room temperature. Antigens were identified by reactions with appropriate antisera following incubation of the plates for 16 hr at room temperature.

Demonstration of *anti-immunoglobulins* was performed by Latex-agglutination (Singer & Plotz, 1956), Waaler-Rose test (Rose *et al.*, 1948) and by agglutination of human Rh+ erythrocytes sensitized by anti-Rh serum 'Ripley' (Waller & Vaughan, 1956).

The presence of *circulating immune complexes* was demonstrated by platelet aggregation (Pl.A.) technique developed by Penttinen & Myllylä (1968). Briefly, human platelets were separated by differential centrifugation from blood containing 0·5% EDTA (Palosuo *et al.*, 1976). Platelets were washed with physiological saline and finally resuspended into a modified Ringer's solution (Myllylä, 1973) to a concentration of 200,000 cells/mm^3. Interaction of platelets with immune complexes was demonstrated by altered sedimentation pattern of the cells during overnight stay in refrigerator. Sera containing Pl.A.-positive material were subjected to sucrose gradient centrifugation to determine the sedimentation properties of the active components.

Quantitative IgG determinations in the fractions were performed by single radial diffusion technique (Mancini, Carbonara & Heremans, 1965).

Pemphigus antibody titre was determined by the method of Beutner & Jordan (1964). The characteristics of the fluorescein-conjugated rabbit antihuman IgG antiserum used in the tests have been previously described (Maize, Dobson & Provost, 1975).

Preparation of tissue antigens

A ubiquitous tissue antigen (to be designated as UTA) was extracted from microsomal fractions of various tissues of human or animal origin by solubilization with 0·5% sodium deoxycholate (DOC) as described elsewhere (Kasukawa *et al.*, 1967). Partial purification of UTA was achieved by fractional precipitation with ammonium sulphate followed by precipitation with ethanol and gel filtration on Sephadex G-200 (Palosuo & Milgrom, 1976). Renal tubular epithelial (RTE) antigen was prepared from the 'FxIA' fractions of normal human kidneys according to Edgington, Glassock & Dixon (1968). Plain saline extracts (30% w/v) and BE antigens (boiling resistant, ethanol insoluble) or various human organs were prepared as described previously (Milgrom & Witebsky, 1962).

A previously described human serum (RH) was used as a reference antiserum for UTA (Kasukawa *et al.*, 1967).

RESULTS

Antibodies to tissue antigens were determined by DDGP tests with DOC extracts of human liver and kidney and rat liver, containing the UTA, RTE antigen preparations of human kidney, plain saline

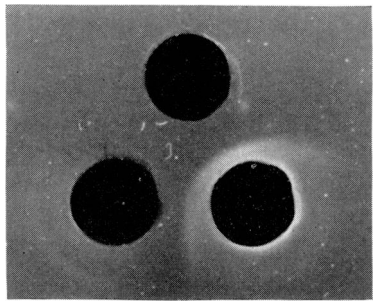

FIG. 1

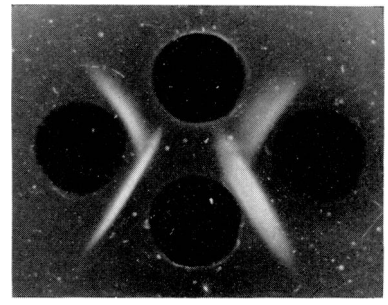

FIG. 2

FIG. 1. Upper well: serum RH. Lower left well: M.M. serum No. 4. Lower right well: UTA-containing serum LM.

FIG. 2. Upper well: serum RH. Lower well: M.M. serum No. 4. Left well: partially purified DOC extract of rat liver. Right well: partially purified DOC extract of human liver.

extracts and BE preparations of various organs. In addition, serum containing the UTA which originated from a patient L.M. with fatal antiglomerular-basement membrane disease, was included in the tests. Serum No. 4 obtained from patient M.M. on 30 January 1974, 4 months after the gold therapy was initiated, reacted with most of the antigens studied. Lines formed by serum No. 4 and reference serum RH with the UTA-containing serum L.M. merged into a reaction of complete identity (Fig. 1). A reaction

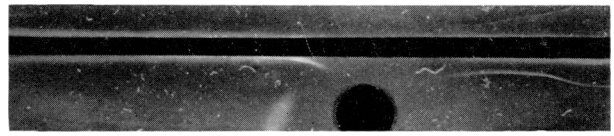

FIG. 3. Immunoelectrophoresis. Circular well: DOC extract of human liver, Horizontal trough: M.M. serum No. 4. Anode is on the left.

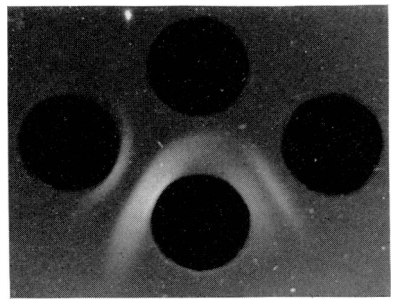

FIG. 4

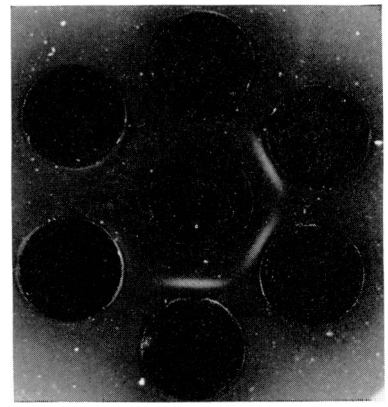

FIG. 5

FIG. 4. Lower well: M.M. serum No. 4. Left well: DOC extract of human liver. Upper well: 30% saline extract of human liver. Right well: BE preparation of human liver.

FIG. 5. Central well: M.M. serum No. 4. Peripheral wells clockwise starting at the top: M.M. sera Nos. 1, 2, 5, 8, 6, 7. (See Table 1 for dates of the sera.)

of partial identity between lines produced by serum No. 4 and serum RH was observed when partially purified DOC extracts (Palosuo & Milgrom, 1976) of human or rat liver were employed as antigens (Fig. 2). However, major reactivity of serum No. 4 with DOC extracts appeared to be directed against an antigenic component with α-globulin electrophoretic mobility (Fig. 3), thus differing from the reaction of the reference anti-UTA serum RH which only detects antigen(s) moving towards the cathode in immuno-electrophoresis (Kasukawa *et al.*, 1967; Palosuo & Milgrom, 1976). The cathodal line seen in the electrophoretogram most likely represents UTA. Serum No. 4 also combined with plain saline extracts and BE preparation of human organs such as kidney and liver, forming one or two partially superimposed precipitation lines. Lines formed by this serum with saline extract, DOC extract, and BE preparation of human liver formed reactions of complete identity (Fig. 4). The line seen close to the well containing the DOC extract most likely represents UTA, while the other lines are likely to represent the α-globulin-like component of the tissue extract. When serum No. 4 was tested against RTE antigen preparation of human kidney and DOC extract of human liver (not shown in the figures), a reaction of complete identity was observed. The shape and the position of the line suggested that UTA was not involved in this reaction. Sera No. 1, 2, 5, 6 and 8 contained a circulating antigen which was precipitated by serum No. 4. This is seen in Fig. 5 in which the lines with sera No. 2, 5, and 8 are quite strong whereas those with sera No. 1 and 6 are very weak. The circulating serum antigen shared determinants with DOC-extracted antigens of human liver (Fig. 6). The strong line of precipitation apparently

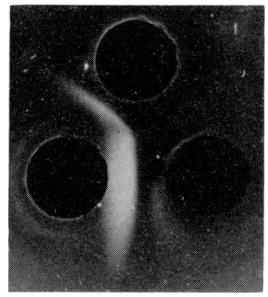

FIG. 6. Upper well: M.M. serum No. 5. Lower left well: M.M. serum No. 4. Lower right well: DOC extract of human liver.

was the α-globulin component of the DOC extract whereas the reaction with UTA could not be clearly noted in this particular experiment. The serum antigen was thermostable, resisting heating to 80°C for 10 min. Serum No. 4 did not react with any of twelve normal human sera tested by means of DDGP.

Circulating immune complexes were demonstrated by the Pl.A. technique. 'Heavy' complexes, sedimenting in sucrose gradients faster than the 19S marker (an infectious mononucleosis serum with high titre of Paul-Bunnell antibodies) were found in serum No. 4 and 12, and intermediate complexes, sedimenting between 19S and 7S markers (7S indicated by quantitative IgG determinations in the fractions), were detected in serum No. 6 and 12 (Fig. 7). The low Pl.A. activities (1:4) in fractions 1–4 of serum No. 6 cannot be considered as significant because such reactions are sometimes seen with similarly fractionated normal human sera. The nature of the very slowly sedimenting Pl.A. active component (<7S) observed in serum No. 4 remains unknown.

Anti-immunoglobulins (rheumatoid factors) as measured by Latex-agglutination, Waaler-Rose test and 'Ripley' tests, were detectable (Table 1). Latex-test was positive with one sample only (serum No. 4). Waaler-Rose reaction was negative (titre <10) at the time when gold therapy was started, reached maximum titres (320) 6 weeks before the onset of proteinuria, and was again negative at the time when proteinuria was noted. An interesting phenomenon was the transitory disappearance of the 'Ripley-reactive' rheumatoid factor at the height of Waaler-Rose titres.

Pemphigus-antibody titres, as followed for several years, had always been in the range of 320–640,

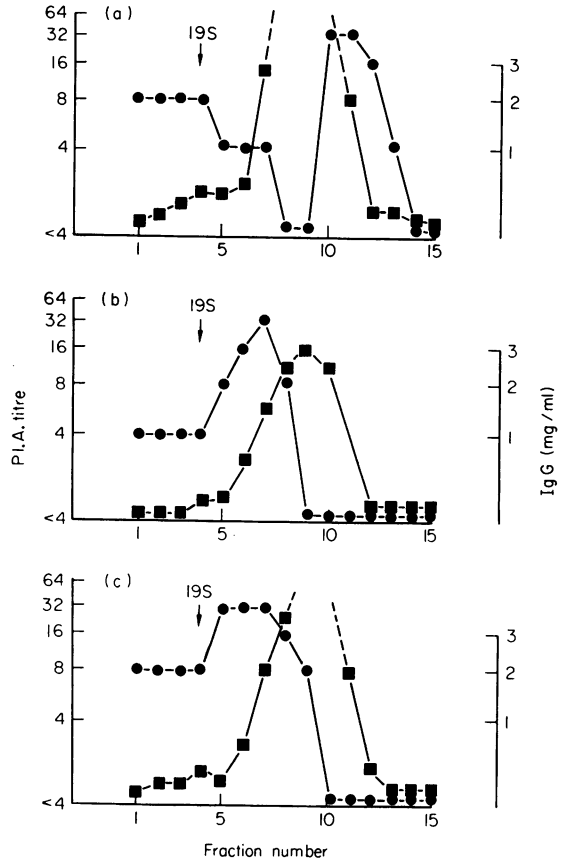

FIG. 7. Sucrose density gradient analyses of three sera from patient M.M. (a) Serum No. 4; (b) serum No. 6; (c) serum No. 12. A sample of 0·5 ml of serum was layered on top of preformed 10–40% (w/v) sucrose gradient in phosphate buffered saline, pH 7·2, and centrifuged in a Spinco SW 50·1 rotor for 16 hr at 30,000 rpm at 4°C. Fractions were collected from below and assayed for Pl.A. activity (●) and IgG content (■).

despite high doses of steroids, methotrexate and azathioprine. Following gold treatment, the titre fell to 40–80, and with re-occurrence of pemphigus lesions, rose again to 320. With further control of the disease with prednisone and azathioprine, the titre is now 40.

DISCUSSION

An autologous immune complex pathogenesis may be anticipated in gold-induced nephropathy. Deposition of immunoglobulins and complement along the glomerular capillary walls has been clearly established (Silverberg *et al.*, 1970) and the ultrastructural findings are typical of an immune complex lesion (Törnroth & Skrifvars, 1974). However, there were no reports concerning the specificity of the complexes involved nor was there any information of possible serological aberrations during this complication of gold treatment. The multitude of immunological phenomena in the present patient, preceding the nephrotic syndrome, was striking. Unfortunately, a renal biopsy was not performed on our patient, and we were not able to investigate the immuno-histology of the kidney lesions.

In a previous publication from this laboratory it was reported that sera and urines of some patients with various renal diseases contained a ubiquitous tissue antigen (designated as UTA), extractable most readily by DOC from microsomal fractions of various organs (Kasukawa *et al.*, 1967). It was found in recent studies that sera from patients with idiopathic membranous nephropathy frequently contained this tissue antigen (Palosuo *et al.*, 1975). Interestingly, serum No. 4 of the present patient reacted with

a serum LM containing this antigen. Furthermore, a circulating serum antigen was demonstrable in several serum specimens which shared antigenic determinants with DOC-extractable tissue antigens. However, considerable part of the 'anti-tissue' antibody activity was directed against an α-globulin-like component of the tissue extract, and not against the UTA in DOC extracts which has a γ-globulin mobility (Kasukawa *et al.*, 1967; Palosuo & Milgrom, 1976).

Serum No. 4 also combined with a preparation of human kidney containing RTE antigens (Edgington *et al.*, 1968); however, the line of precipitation showed a reaction of complete identity with that formed between a DOC extract of human liver and the same serum. Thus, this could hardly indicate a reaction with a kidney-specific antigen. On the other hand, precipitating antibodies to the RTE antigens have not been reported to our knowledge in human patients.

Precipitating antibodies to saline extracts of human organs have been frequently found in sera of patients with systemic lupus erythematosus (Miescher & Vorlaender, 1961; Tan & Kunkel, 1966; Clark, Reichlin & Tomasi, 1969), but antibodies to BE antigens have not been described in human sera. Serum No. 4 of the present patient contained such antibodies; however, neither this serum nor other serum samples of this patient agglutinated human erythrocytes treated with tannic acid and coated with these antigens. Further studies into the nature of these antibodies are clearly necessary.

Circulating immune complexes were detected in three sera by means of Pl.A. combined with sucrose gradient centrifugation. In addition to circulating immune complexes, positive reactions obtained with this technique can be due to non-specifically aggregated IgG and anti-platelet antibodies (Penttinen *et al.*, 1973). Special care was taken to handle properly serum samples of the patient. They were stored at −20°C and actually thawed for the second time when used for sucrose gradient analysis. It is thus likely that the observed reactions were due to immune complexes. It is especially unlikely that the Pl.A.-active components sedimenting at 'intermediate speed' in sucrose gradients were due to artificially aggregated IgG, since the latter type of activities have always had 'heavy' (⩾19S) component-characteristics (Penttinen *et al.*, 1973). Our attempts to elucidate the immunologic specificity of the complexes by adding various antigens to the serum in order to produce antigen excess and disappearance of the Pl.A. reaction (Penttinen, Vaheri & Myllylä, 1971; Myllylä, Vaheri & Penttinen, 1971) were unsuccessful.

Rheumatoid factors were detected in several sera of our patient. The disappearance of 'Ripley-reactive' factors at the time when Waaler-Rose titres were at their highest and circulating immune complexes demonstrable was especially interesting. One may postulate that these 'human-specific' anti-immunoglobulins were bound to immune complexes while considerable amounts of Waaler-Rose-reactive factors, of broader specificity, remained in circulation.

Parenteral administration of gold sodium thiomalate was recently introduced as an alternate therapy for treatment of pemphigus vulgaris (Penneys *et al.*, 1973). Our observation is similar to those of Penneys *et al.* (1973) who found gold therapy successful in the control of the disease. Healing of pemphigus lesions was associated with a significant fall in pemphigus antibody titre also in our patient and after discontinuation of gold the titre rose again.

It cannot be stated on the basis of our results whether the observed immunological phenomena were causally related to the development of the nephrotic syndrome of our patient. The rapid disappearance of tissue antibodies prior to the onset of the nephropathy speaks in favour of the idea that the antibodies were bound to corresponding target antigens in organs or were deposited in the glomeruli as immune complexes. Studies are in progress to find out how frequently tissue antibody response occurs during chrysotherapy and whether it is linked to precipitation of glomerulonephritis. The latter possibility would provide prognostic significance to screening of tissue antibodies in patients receiving treatment with gold salts and possibly during exposure to other heavy metals.

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